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Full Length Research Paper

Evaluation of bacterial culture and their resistant pattern in pus containing patients of different wards of the hospital, Lahore, Pakistan

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In health care delivery worldwide, infections acquired in health care setting are the most challenging factors. Infections due to antibiotic resistant bacteria have increased day by day in both developed and developing countries. This study is performed to screen the bacterial pathogens present in pus samples taken from patients of different wards of hospital. Isolation of bacteria was determined by standard microbial techniques. Total number of samples is 383 in which 252 were males and 131 were females. 265 samples have growth (259 were suitable for culturing and other 6 were not suitable for culturing) and 118 samples have no growth. Most samples have single growth, while some samples have mixed growth. The predominant genera were coliform including *Escherichia coli*, *Klebsiella*, *Pseudomonas*. *Staphylococcus aureus* has small number of cultures. These bacterial pathogens show resistant to most of the commonly used antibiotics. The resistant pattern was high in both gram negative and gram positive bacteria. Some organisms show multi-drug resistant pattern.

Key words: No growth, bacterial, patients, genera.

INTRODUCTION

Infectious diseases still remain an important cause of morbidity and mortality among humans, especially in developing countries. Skin abrasion due to surgical procedure, trauma, burns, accidental cases, nutrition and other factors affects this first line defense and leads to microbial contamination causing infections and infectious diseases. From different local hospital wards bacterial isolates and their antibiotic susceptibility patterns among patients with pus and/or wound discharge were evaluated.

Various species of bacteria live on human skin, gastrointestinal tract, in the nasopharynx and other parts of the body with less potential for causing disease because of first line defense within the body. Wound is a type of injury in which skin is torn, cut, or punctured or where blunt force trauma causes a closed wound. In pathology, it specifically refers to a sharp injury which damages the upper layer of the skin. The concept of bacterial burden in these wounds has been established

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over a number of years, and modulation of the wound microflora and bacterial biofilm is an important treatment-aim in the management of patients with wound. This treatment may be affected by antimicrobial dressings, antiseptics and the prescription of topical or systemic antibiotics (Muluye et al., 2013).

Pus is protein-rich fluid called *liquor puris*, usually whitish-yellow, yellow, or yellow brown in color. Pus consists of a buildup of dead leukocytes (white blood cells) from the body's immune system in response to infection. Pus forming bacteria are called Pyogenic (Madigan and Martin, 2006). Names of bacteria which form pus (Pyogenic) are *Staphylococcus aureus*, *Staphylococcus Epidermidis, Streptococcus pyogenes, Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa, Neisseria gonorrhoeae, Actinomyces, Burkholderia mallei, Mycobacterium tuberculosis.* These include both Gram positive and Gram negative bacteria. *S. aureus, Enterobacteriaceae* and *Pseudomonas* species have potentially adverse effects on wound healing (Roy et al., 2017).

Antibiotic susceptibility testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection *in vivo*. Antibiotics are the main options for curing diseases. Selection of an effective antimicrobial agent for a microbial infection requires knowledge of the potential microbial pathogen, an understanding of the pathophysiology of the infectious process and an understanding of the pharmacology and pharmaco kinetics of the intended therapeutic agents (Getachew et al., 2018).

MATERIALS AND METHODS

Bacterial assay

A cross sectional study was done in Sheikh Zaid Hospital Lahore from 20 February, 2014 to 10 June, 2014. Data of 385 patients were collected during this time period from different wards of hospital. Samples from open wound were collected by sterile swabs. All these samples were transported to lab in sterile containers within 30 min of sample collection. Different types of media (differential and selective) were used for inoculation. After pouring this media on plate it was left to solidify. Preliminary identification of bacteria was based on colony characteristics of the organisms. In the differential media there was haemolysis of blood agar, chocolate agar, MacConkey agar at 37°C for 24 h and changes in their physical appearance. Gram staining was done for further identification of their shape (cocci, rod, and coccobacilli). Biochemical test was done including catalase, coagulase, oxidase, urea and motility.

Antibiotic assay

Susceptibility testing was performed by Kirby-Bauer disk diffusion technique. The test organism was uniformly seeded over the Mueller-Hinton agar surface and exposed to a concentration gradient of antibiotic diffusing from antibiotic-impregnated paper disk into the agar medium, and then incubated at 37°C for 18-24 h. Diameters of the zone of inhibition around the discs were measured to the nearest millimeter using a ruler and classified as sensitive, intermediate, and resistant according to the standardized table.

The drugs tested for both gram negative and positive bacteria were Ampicilin (Amp,10 µg), Erythromycin (E, 15 µg), Amikacin (AK, 30 µg), Vancomycin (VA, 30 µg), Penicillin (P, 10 µg), Oxacillin (OX,1 µg), Imipenem (Ipm,10 µg), Gentamycin (CN, 10 µg), Ciprofloxacin (Cip, 5 µg), Amoxicillin (AMX, 20 µg), Linezolid (LZ, 30 µg), Cefotaxime (CTX, 10 µg), Polymyxin B (PB, 50 µg),Ceftazidime (CAZ, 30 µg). Ceftriaxone (CRO, 10 µg), Amoxyclav (AMC,10 µg), Cefuroxine (CXM,30 µg), Piperacillin (PIT,10 µg),Tetracycline (TEC,30 µg),Meropenem(MEM,10 µg), Tazobactum (TZP,10 µg), Cephalotin (CE,30 µg).

Data analysis

Data were cleaned manually and analyzed using SPSS version 20 software. Odds ratio and Chi-square test were employed. P-value ≤ 0.05 was considered statistically significant

RESULTS

A total of 385 wound patients specimens were collected from different hospital wards. Of these 385, 131(34.20%) were females and 252 (65.80%) were males. Samples which were not suitable for culturing were 6 (1.30%). Samples which have no growth were 118(31%). Samples which have growth were 259 (67.80%). Samples which have single growth were 221 (85.30%) out of 259. Samples having mixed or multiple growth are 38 (14.70%) out of 259. Total numbers of bacteria isolated were 298 (Table 1 and Figure 1).

The results obtained show that the organisms varied in their susceptibility to all the antimicrobial used. Majority of them showed multi-resistances (resistance to two or more classes of antimicrobial); 98% organisms showed multi-resistant pattern.

The antibiotics which were used in lab for testing were frequently available and prescribed by doctors. Total 25 of antibiotics of 6 different classes were used. The results showed by these antibiotics were: for CX used in 181 organisms, 100(55.20%) showed sensitivity and 81(44.80%) showed resistance. For Cip used in 179 samples, 55 (30.80%) were sensitive and 124 (69.20%) were resistant. For E used in 99 samples (14.14%) were sensitive and 85(85.86%) were resistant. For CAZ used in 170 samples 25 (14.70%) were sensitive and 145 (85.70%) were resistant. For AMX used in 9 samples 1(11.20%) were sensitive and 8 (88.80%) were resistant. For PB used in 4 samples 3 (75%) were sensitive and 1(25%) were resistant. For OX used in 4 samples 2 (50%) were sensitive and 2(50%) were resistant. For PIT used in 2 samples 1 (50%) was sensitive and 1 (50%) were resistant (Table 2).

DISCUSSION

There were 385 samples examined that yield 259(68%)

Table 1. Bacterial isolate with hospital ward.

Name of bacteria	Surgical	Orthopedic	ICU	Nephrology	LTU	ENT	OPD	MED	FUW	GS	PW	MNW
Coliform	35	9	10	9	12	4	3	7	4	6	3	4
Pseudomonas	6	1	2		2	8	1		1	2	1	1
Candida	1	1	1	1	4	1		1	1		1	
Staph aureus	5	10	0	3	1	2	5	3	2		2	
Proteus	2	1	1	2		1	1	1				
Acinetobacter	1	0	3	1			2					
Enterococci	3	0	0	1	1				1			1
Klebsiella	0	1	0									
E. coli			1									
Fungal growth					1					1		
Staphylococcus									1			

ICU, Intensive care unit; LTU, liver transplant unit; ENT, ear nose throat; OPD, outpatient department; MED, Medical ward; GS, general surgery; PW, pediatrics ward; FUW, female unit wards, MNW, male ward.

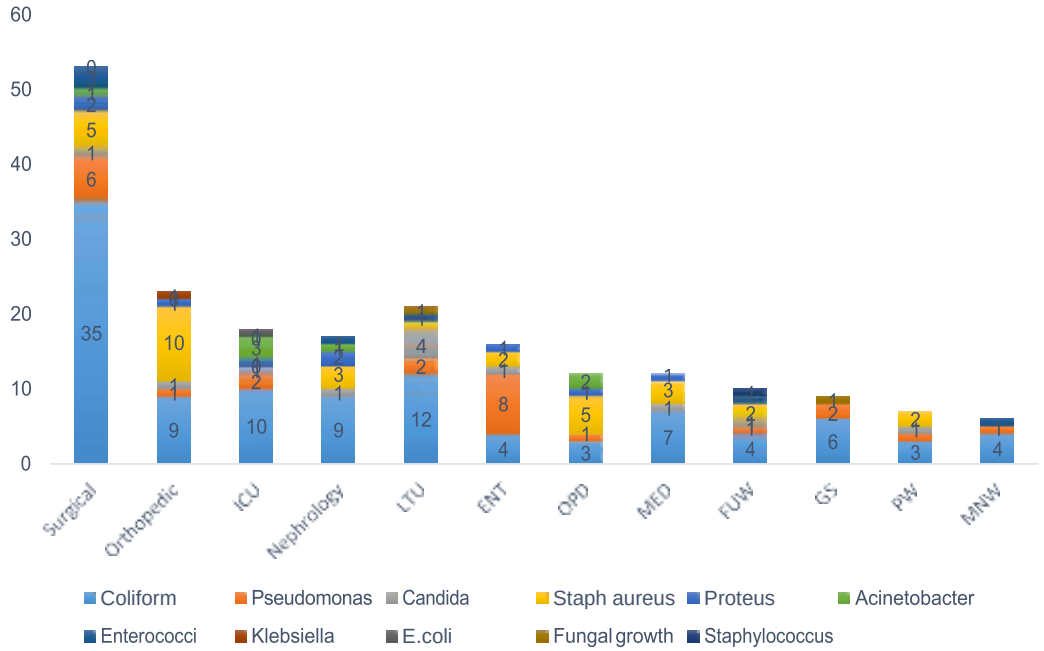

Figure 1. Graphical representation of no of bacterial isolate in different ward of hospital.

Table 2. Sensitivity patterns of organisms against drugs.

Antibiotics	*Coliform*	*S. aureus*	*Pseudomonas*	*Proteus*	*Acineto*	*Enterococcus*	*Staphylococcus*	*Streptococci*	*E. coli*	*Klebsiella*
CX	0	4 (75%)	0	0	0	0	1 (25%)	0	0	0
Cip	18 (32.72%)	19 (35.54%)	12 (21. 815)	0	0	4 (7.27%)	1 (1.81%)	1 (1.81%)	0	0
E	0	14 (100%)	0	0	0	0	0	0	0	0
CAZ	13 (52%)	1 (4%)	8 (32%)	2 (8%)	0	0	0	0	1 (4%)	0
CN	29 (54.7%)	15 (28.03%)	4 (7.54%)	0	3 (5.6%)	0	1 (1.89%)	0	1 (1.89%)	0
TZP	58 (64.44%)	2 (2.22%)	18 (20%)	10 (11.11%)	1 (1.11%)	0	0	0	0	1 (1.11%)
CTX	9 (39.13%)	7 (30.4%)	0	3 (13. 04%)	1 (4.345)	1 (4.34%)	1 (4.34%)	1 (4.3	0	0
AK	58 (74.35%)	4 (5.12%)	12 (15. 38%)	1 (1.28%)	2 (2.56%)	0	0	0	0	1 (1.28%)
Ipm	73 (64.6%)	3 (2.65%)	24 (21. 23%)	11 (9. 73%)	1 (0.88%)	0	0	0	1 (0.88%)	1 (0.88%)
SCF	45 (62.5%)	1 (1.38%)	17 (23. 61%)	9 (12.	0	0	0	0	0	1 (1.38%)
AMC	2 (40%)	0	0	1 (20)	0	1 (20%)	1 (20%)	0	0	0
AMP	2 (28.5%)	1 (14.28%)	0	0	0	1 (14.28)	2 (28.5%)	1 (14.2%)	0	0
P	0	1 (33.33%)	1 (33.33%)	0	0	0	1 (33.33%)	0	0	0
CXM	3 (21.42%)	8 (57.14%)	1 (7.1%)	0	0	0	1 (7.1%)	1 (7.1%)	0	0
MEM	49 (79%)	0	9 (14.51%)	4 (6.45%)	0	0	0	0	0	0
VA	0	34 (69.38%)	0	0	0	10 (20.40%)	4 (8.16%)	1 (2.04%)	0	0
CE	0	6 (85.7%)	0	0	0	0	0	1 (14.2)	0	0
FA	0	33 (78.57%)	0	0	0	4 (9.52%)	5 (11.90%)	0	0	0
Imp	14 (87.5%)	0	1 (7.14%)	1 (7.14%)	0	0	0	0	0	0
CRO	0	2 (67)	0	0	0	0	1 (33%)	0	0	0
LZ	1 (7.14%)	11 (78.57%)	0	0	0	2 (14.28%)	0	0	0	0
AMX	0	0	0	0	0	0	1 (100%)	0	0	0
TEC	0	3 (50%)	0	0	0	3 (50%)	0	0	0	0
PB	3 (100%)	0	0	0	0	0	0	0	0	0
OX	0	2 (100%)	0	0	0	0	0	0	0	0
PIT	1 (100%)	0	0	0	0	0	0	0	0	0

isolates. That means that some samples yielded more than one organisms (polymicrobial) including *Coliform, S. aureus, Klebsiella* and *E-coli;* few samples failed to yield any growth. The predominance of mono-microbial infections in this study has been substantiated by a prospective study. In this study, it was found that 54.40% were *coliform*, 16.60% were *S. aureus*, 13.90% were *P. aeruginosa*, 6.90% were *Proteus*, 6.10% were *Acinetobacter*, 5.79% were *Candida*, 5.01% were *Enterococcus*, 1.90% were *Staphylococcus* spp., 1.54% were *Coliform* (nlf), 1.15% were fungal growth, 0.76% were *Streptococcus*, 0.38% were *E. coli* and 0.38% were *Klebsiella.* Similar results were shown in the report of Manikandan and Amsath (2013) that *S. aureus* (24.3%) were the most common organisms followed by *Staphylococcus* spp. (15.7%), *Proteus* spp. (8.6%), *E. coli* (5.7%) and *Klebsiella* (2.8%). Similarly, Godebo et al. (2013) reported 77% isolation rate of *Staph aureus*. Again similar finding of Verma and Chandrakar (2012) reported 40% isolation rate of *S. aureus* and has high resistant (11.64%); also the finding shows that

33.1% were *S. aureus,* suggesting that the present work has similarity against isolated bacteria and sensitivity to ceftrioxine is more compared to other antibiotics against *S. aureus.* Similarly, in previous work they were resistant to methicillin but all sensitive to Vancomycin (Javeed et al., 2011). Similarly Roy et al. (2017) stated that 59.1% for cotrimoxazole and 66.2% resistant to tetracycline but in present study resistant to amoxicillin is 88.8% while resistant to ceftrizidime is 85%. Getachew et al. (2018) reported that resistance of *S. aureus to* methicillin continuously increased day by day and also show multi-drug resistant pattern against common antibiotics. Similarly the present study has shown more resistant against *S. aureus* and also multi-drug resistance against six different broad classes of antibiotics.

Finally, the pus samples collected from patients of different wards of hospital show mono microbial growth and have a high yield of Gram negative bacteria which were multiple drugs resistant. The high isolation rate of gram negative is due to unhygienic conditions. A number of bacteria show resistant to commonly given antibiotics of different classes and small numbers of them show sensitivity to these drugs. Due to their resistant it is difficult to cure infection.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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